

PATENT CLAIMS

What we claim is:

1. A subtilase variant having improved storage stability and/or improved performance in detergents, wherein one or more amino acid residues situated in, or in the vicinity of a hydrophobic domain of the parent subtilase have been substituted for an amino acid residue more hydrophobic than the original residue, said hydrophobic domain comprising the residues corresponding to residues P129, P131, I165, Y167, Y171 of BLS309 (in BASBPN numbering), and said residues in the vicinity thereof comprises residues corresponding to the residues E136, G159, S164, R170, A194, and G195 of BLS309 (in BASBPN numbering), with the exception of the R170M, R170I and R170V variants of BABP92 and the R170Y variant of BLS309.
2. The variant of claim 1, wherein said variant exhibits improved stability in liquid detergents.
3. The variant of claim 1, wherein said variant exhibits improved stability in detergents in shaped solid form.
4. The variant of claim 1, wherein said variant exhibits improved wash performance.
5. The variant of claim 1, wherein the original amino acid residue has been substituted for any other residue which increase the hydrophobicity of the original residue, where the substituted residue preferably is a residue selected from the group comprising Val (V), Ile (I), Leu (L), Met (M), Phe (F), Pro (P), and Trp(W), especially Val, Ile or Leu.
6. The variant of claim 1, wherein the parent subtilase is chosen from the sub-group I-S1.
7. The variant of claim 6, wherein the parent subtilase is chosen from the group comprising ABSS168, BASBPN, BSSDY, and BLSCAR.
8. The variant of claim 1, wherein the parent subtilase is chosen from the sub-group I-S2.
9. The variant of claim 8, wherein the parent subtilase is chosen from the group comprising BLS147, BLS309, BAPB92 AND BYSYAB.

10. The variant of claim 8, wherein the parent subtilase is TVTHER.
11. The variant of claim 1, wherein said substitution(s) is/are combined with substitutions, insertions or deletions in any other position.
12. The variant of claim 11, wherein said substitution(s) is/are combined with substitutions, insertions or deletions in any of the positions 36, 222, 218, 76.
13. The variant of claim 1, being any of the following:
T129V, T129I, T129L, T129M, T129F
A129V, A129I, A129L, A129M, A129F
G131V, G131I, G131L, G131M, G131F
K136V, K136I, K136L, K136M, K136F,
S159V, S159I, S159L, S159M, S159F,
T164V, T164I, T164L, T164M, T164F,
K170V, K170I, K170L, K170M, K170F,
Q136V, Q136I, Q136L, Q136M, Q136F,
T159V, T159I, T159L, T159M, T159F,
A164V, A164I, A164L, A164M, A164F,
Y170V, Y170I, Y170L, Y170M, Y170F,
Y171A, Y171H, Y171N, Y171P, Y171C, Y171W,
Y171Q, Y171S, Y171T, Y171G
Y171V, Y171I, Y171L, Y171M, Y171F
S194V, S194I, S194L, S194M, S194F,
E136V, E136I, E136L, E136M, E136F,
G159V, G159I, G159L, G159M, G159F,
G164V, G164I, G164L, G164M, G164F,
S164V, S164I, S164L, S164M, S164F,
Y167A, Y167H, Y167N, Y167P, Y167C, Y167W, Y167Q, Y167S, Y167T, Y167G, Y167V,
Y167I, Y167L, Y167M, Y167F,
R170A, R170H, R170N, R170P, R170C, R170W, R170Q, R170S, R170T, R170Y, R170G,
R170V, R170I, R170L, R170M, R170F,
A194V, A194I, A194L, A194M, A194F,

P194V, P194I, P194L, P194M, P194F,
E195V, E195I, E195L, E195M, E195F,
G195V, G195I, G195L, G195M, G195F,

14. The variant of claim 1, wherein said variant is combined with further substitutions, deletions and/or insertions in any one or more of the positions: 27, 36, 57, 76, 97, 101, 104, 120, 123, 206, 218, 222, 224, 235 and 274.

15. The variant of claim 14, wherein said subtilase belongs to the I-S2 sub-group and said further change is chosen from the group comprising K27R, *36D, S57P, N76D, G97N, S101G, V104A, V104N, V104Y, H120D, N123S, A194P, Q206E, N218S, M222S, M222A, T224S, K235L, and T274A.

16. The variant of claim 15 comprising any one or two of the substitutions X167V, X167M, X167F, X167L, X167I, X170V, X170M, X170F, X170L, and/or X170I in combination with any one or more of the other substitutions, deletions and/or insertions mentioned in any of claims 1 to 15.

17. The variant of claim 15 further comprising any of the variants V104N+S101G, K27R+V104Y+N123S+T274A, or N76D+V104A, or other combinations of these mutations (V104N, S101G, K27R, V104Y, N123S, T274A, N76D, V104A).

18. The variant of claim 15, selected from the group consisting of:

- a) S57P+R170L
- a') S57P+R170I
- b) R170L+N218S
- b') R170I+N218S
- c) S57P+R170L+N218S
- c') S57P+R170I+N218S
- c'') S57P+V104Y+R170L+N218S
- c''') S57P+V104Y+R170I+N218S
- d) R170L+N218S+M222A
- d') R170I+N218S+M222S
- d'') R170L+N218S+M222A
- d''') R170I+N218S+M222S

- e) S57P+R170L+S188P+A194P
- e') S57P+R170I+S188P+A194P
- f) Y167L+R170L
- f') Y167L+R170I
- g) Y167I+R170L
- g') Y167I+R170I
- h) N76D+R170L+N218S
- h') N76D+R170I+N218S
- i) S57P+N76D+R170L+N218S
- i') S57P+N76D+R170I+N218S
- j) N76D+R170L+N218S+M222A
- j') N76D+R170I+N218S+M222S
- j'') N76D+R170L+N218S+M222A
- j''') N76D+R170L+N218S+M222S
- k) S57P+R170I+S188P+A194P+N218S
- k') S57P+R170I+S188P+A194P+N218S
- l) *36D+N76D+H120D+R170L+G195E+K235L
- l') *36D+N76D+H120D+R170I+G195E+K235L
- l'') *36D+N76D+H120D+Y167I+R170L+G195E+K235L
- l''') *36D+N76D+H120D+Y167I+R170I+G195E+K235L
- m) N76D+H120D+R170L+G195E+K235L
- m') N76D+H120D+R170I+G195E+K235L
- m'') N76D+H120D+Y167I+R170L+G195E+K235L
- m''') N76D+H120D+Y167I+R170I+G195E+K235L
- n) *36D+G97N+V104Y+H120D+R170L+A194P+G195E+K235L
- n') *36D+G97N+V104Y+H120D+R170I+A194P+G195E+K235L
- o) S57P+R170L+Q206E
- o') S57P+R170I+Q206E
- p) R170L+Q206E
- p') R170I+Q206E
- q) Y167I+R170L+Q206E
- q') Y167I+R170I+Q206E
- r) Y167F+R170L
- r') Y167F+R170I

- t) Y167I+R170L+A194P
- t') Y167I+R170I+A194P
- t'') Y167L+R170L+A194P
- t''') Y167L+R170I+A194P
- u) Y167I+R170L+N218S
- u') Y167I+R170I+N218S
- u'') Y167L+R170L+N218S
- u''') Y167L+R170I+N218S
- v) Y167I+R170L+A194P+N218S
- v') Y167I+R170I+A194P+N218S
- v'') Y167L+R170L+A194P+N218S
- v''') Y167L+R170I+A194P+N218S
- x) R170L+P131V
- x') R170I+P131V
- y) *36D+Y167I+R170L
- y') *36D+Y167I+R170I
- z) Y167I+Y171I
- aa) Y167V+R170L
- aa') Y167V+R170I
- bb) R170L+Y171I
- bb') R170I+Y171L
- bb'') R170L+Y171L
- bb''') R170I+Y171I
- cc) Y167I+Y171L+N218S
- cc') Y167I+Y171I+N218S

19. A process which comprises effecting a mutation in DNA encoding a subtilase enzyme or its pre- or proenzyme at one or more of the positions corresponding amino acid residues in or in the vicinity of a hydrophobic domain of the parent subtilase have been substituted for an amino acid residue more hydrophobic than the original residue, said hydrophobic domain comprising the residues P129, P131, I165, Y167, Y171 of BLS309, and the residues in the vicinity thereof for BLS309 are E136, G159, S164, R170, A194, and G195, with the exception of the R170M, R170I and R170V variants of BABP92, and testing for improved storage stability and/or wash performance in detergents.

20. A process which comprises the manufacture of a mutant subtilase enzyme having a desired improved storage stability or wash performance following its identification according to the procedure of claim 19.

21. A DNA sequence encoding a subtilase variant of claim 1.

22. A vector comprising a DNA sequence of claim 21.

23. A microbial host transformed with a vector of claim 22.

24. The microbial host of claim 23, which is a bacterium, preferably a *Bacillus*.

25. The microbial host of claim 23, which is a fungus or yeast, preferably a filamentous fungus, especially an *Aspergillus*.

26. A method for producing a variant of claim 1, comprising culturing a host transformed with a vector comprising a DNA sequence encoding the variant under conditions conducive to the expression and secretion of said variant, and recovering the variant.

27. A detergent composition comprising a subtilase variant according to claim 1 and a surfactant.

28. The detergent composition of claim 27, wherein said composition is in solid powdered form.

29. The detergent composition of claim 27, wherein said composition is in shaped solid form.

30. The detergent composition of claim 27, wherein said composition is in liquid form.

31. The detergent composition of claim 30, wherein said liquid is a structured liquid.

32. The detergent composition according to claim 27, wherein the subtilase variant is R170L or R170I and the composition is a liquid detergent and comprises a deflocculating polymer.

33. The detergent composition according to claim 27, wherein the subtilase variant is S57P+V104Y+R170L+N218S or S57P+V104Y+R170I+N218S.

34. A modified subtilase comprising a substitution selected from the group consisting of:

- (a) a substitution of the amino acid residue at position 167 with Met, Phe, Pro, Trp, or Val, and
- (b) a substitution of the amino acid residue at position 170 with Ile, Met, or Val,
- (c) a substitution of the amino acid residue at position 171 with Met, Phe, Pro or Trp,
- (d) a substitution of the amino acid residue at position 194 with Ile, Met, Phe, Pro, Trp, or Val,

wherein each position corresponds to a position of the amino acid sequence of subtilisin BPN'.

35. The modified subtilase of claim 34, which comprises a substitution of the amino acid residue at position 167 with Met, Phe, Pro, Trp, or Val.

36. The modified subtilase of claim 34, which comprises a substitution of the amino acid residue at position 170 with Ile, Met, or Val.

37. The modified subtilase of claim 34, which comprises a substitution of the amino acid residue at position 171 with Met, Phe, Pro or Trp.

38. The modified subtilase of claim 34, which comprises a substitution of the amino acid residue at position 194 with Ile, Met, Phe, Pro, Trp, or Val.

39. The modified subtilase of claim 34, further comprising at least one further mutation at one or more of positions: 27, 36, 57, 76, 97, 101, 104, 120, 123, 206, 218, 222, 224, 235 and 274.

40. The modified subtilase of claim 39, wherein at least one further mutation is selected from the group consisting of K27R, *36D, S57P, N76D, G97N, S101G, V104A, V104N, V104Y, H120D, N123S, Q206E, N218S, M222A, M222S, T224S, K235L, and T274A.

41. A detergent composition comprising a modified subtilase of claim 34 and a surfactant.

42. An isolated nucleic acid encoding a modified subtilase of claim 34.

43. A vector comprising a nucleic acid of claim 42.
44. A microbial host cell comprising a vector of claim 43.
45. A method for producing a modified subtilase, which comprises
- (a) culturing a microbial host cell of claim 44 under conditions conducive to the expression and secretion of the modified subtilase, and
 - (b) recovering the modified subtilase.
46. A modified subtilisin 309 comprising a substitution selected from the group consisting of:
- (a) a substitution of Glu at position 136 with Ala, Asn, Cys, Gln, Gly, His, Ser, Thr, or Tyr,
 - (b) Y171A, Y171C, Y171F, Y171G, Y171H, Y171M, Y171N, Y171P, Y171Q, Y171S, Y171T, or Y171W,
- wherein each position corresponds to a position of the amino acid sequence of subtilisin BPN'.
47. The modified subtilisin 309 of claim 46, which comprises a substitution of Glu at position 136 with Ala, Asn, Cys, Gln, Gly, His, Ser, Thr, or Tyr.
48. The modified subtilisin 309 of claim 46, which comprises Y171A, Y171C, Y171F, Y171G, Y171H, Y171M, Y171N, Y171P, Y171Q, Y171S, Y171T, or Y171W.
49. The modified subtilisin 309 of claim 46, further comprising at least one further mutation at one or more of positions: 27, 36, 57, 76, 97, 101, 104, 120, 123, 194, 206, 218, 222, 224, 235 and 274.
50. The modified subtilisin 309 of claim 49, wherein at least one further mutation is selected from the group consisting of K27R, *36D, S57P, N76D, G97N, S101G, V104A, V104N, V104Y, H120D, N123S, A194P, Q206E, N218S, M222A, M222S, T224S, K235L, and T274A.
51. A detergent composition comprising a modified subtilisin 309 of claim 46 and a surfactant.
52. An isolated nucleic acid encoding a modified subtilisin 309 of claim 46.
53. A vector comprising a nucleic acid of claim 52.

54. A microbial host cell comprising a vector of claim 53.
55. A method for producing a modified subtilisin 309, which comprises
- (a) culturing a microbial host cell of claim 54 under conditions conducive to the expression and secretion of the modified subtilisin 309, and
 - (b) recovering the modified subtilisin 309.
56. A modified subtilisin 147 comprising a substitution selected from the group consisting of:
- (a) a substitution of Glu at position 136 with Ala, Asn, Cys, Gln, Gly, His, Ser, Thr, or Tyr,
 - (b) Y171A, Y171C, Y171F, Y171G, Y171H, Y171M, Y171N, Y171P, Y171Q, Y171S, Y171T, or Y171W,
- wherein each position corresponds to a position of the amino acid sequence of subtilisin BPN¹.
57. The modified subtilisin 147 of claim 56, further comprising at least one further mutation at one or more of positions: 27, 36, 57, 76, 97, 101, 104, 120, 123, 194, 206, 218, 222, 224, 235 and 274.
58. The modified subtilisin 147 of claim 57, wherein at least one further mutation is selected from the group consisting of K27R, *36D, S57P, N76D, G97N, S101G, V104A, V104N, V104Y, H120D, N123S, A194P, Q206E, N218S, M222A, M222S, T224S, K235L, and T274A.
59. A detergent composition comprising a modified subtilisin 147 of claim 56 and a surfactant.
60. An isolated nucleic acid encoding a modified subtilisin 147 of claim 56.
61. A vector comprising a nucleic acid of claim 60.
62. A microbial host cell comprising a vector of claim 61.
63. A method for producing a modified subtilisin 147, which comprises
- (a) culturing a microbial host cell of claim 62 under conditions conducive to the expression and secretion of the modified subtilisin 147, and
 - (b) recovering the modified subtilisin 147.

64. A modified *Bacillus* protease PB92 comprising Y171A, Y171C, Y171F, Y171G, Y171H, Y171M, Y171N, Y171P, Y171Q, Y171S, Y171T, or Y171W, wherein each position corresponds to a position of the amino acid sequence of subtilisin BPN'.
65. The modified *Bacillus* protease PB92 of claim 64, further comprising at least one further mutation at one or more of positions: 27, 36, 57, 76, 97, 101, 104, 120, 123, 194, 206, 218, 222, 224, 235 and 274.
66. The modified *Bacillus* protease PB92 of claim 65, wherein at least one further mutation is selected from the group consisting of K27R, *36D, S57P, N76D, G97N, S101G, V104A, V104N, V104Y, H120D, N123S, A194P, Q206E, N218S, M222A, M222S, T224S, K235L, and T274A.
67. A detergent composition comprising a modified *Bacillus* protease PB92 of claim 64 and a surfactant.
68. An isolated nucleic acid encoding a modified *Bacillus* protease PB92 of claim 64.
69. A vector comprising a nucleic acid of claim 68.
70. A microbial host cell comprising a vector of claim 69.
71. A method for producing a modified *Bacillus* protease PB92, which comprises
- (a) culturing a microbial host cell of claim 70 under conditions conducive to the expression and secretion of the modified *Bacillus* protease PB92, and
 - (b) recovering the modified *Bacillus* protease PB92.
72. A modified subtilisin BPN' comprising a substitution of Lys at position 136 with Ala, Asn, Cys, Gln, Gly, His, Ser, Thr, or Tyr, wherein each position corresponds to a position of the amino acid sequence of subtilisin BPN'.
73. The modified subtilisin BPN' of claim 72, further comprising at least one further mutation at one or more of positions: 27, 36, 57, 76, 97, 101, 104, 120, 123, 194, 206, 218, 222, 224, 235 and 274.

74. The modified subtilisin BPN' of claim 73, wherein at least one further mutation is selected from the group consisting of K27R, *36D, S57P, N76D, G97N, S101G, V104A, V104N, V104Y, H120D, N123S, A194P, Q206E, N218S, M222A, M222S, T224S, K235L, and T274A.
75. A detergent composition comprising a modified subtilisin BPN' of claim 72 and a surfactant.
76. An isolated nucleic acid encoding a modified subtilisin BPN' of claim 72.
77. A vector comprising a nucleic acid of claim 76.
78. A microbial host cell comprising a vector of claim 77.
79. A method for producing a modified subtilisin BPN', which comprises
(a) culturing a microbial host cell of claim 78 under conditions conducive to the expression and secretion of the modified subtilisin BPN', and
(b) recovering the modified subtilisin BPN'.
80. A modified subtilisin Carlsberg comprising a substitution of Lys at position 136 with Ala, Asn, Cys, Gln, Gly, His, Ser, Thr, or Tyr, wherein the position corresponds to a position of the amino acid sequence of subtilisin BPN'.
81. The modified subtilisin Carlsberg of claim 80, further comprising at least one further mutation at one or more of positions: 27, 36, 57, 76, 97, 101, 104, 120, 123, 194, 206, 218, 222, 224, 235 and 274.
82. The modified subtilisin Carlsberg of claim 81, wherein at least one further mutation is selected from the group consisting of K27R, *36D, S57P, N76D, G97N, S101G, V104A, V104N, V104Y, H120D, N123S, A194P, Q206E, N218S, M222A, M222S, T224S, K235L, and T274A.
83. A detergent composition comprising a modified subtilisin Carlsberg of claim 80 and a surfactant.
84. An isolated nucleic acid encoding a modified subtilisin Carlsberg of claim 80.

85. A vector comprising a nucleic acid of claim 84.
86. A microbial host cell comprising a vector of claim 85.
87. A method for producing a modified subtilisin Carlsberg, which comprises
- (a) culturing a microbial host cell of claim 86 under conditions conducive to the expression and secretion of the modified subtilisin Carlsberg, and
 - (b) recovering the modified subtilisin Carlsberg.